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Preservative Effect of Edible Chitosan Coated Liposomes Loaded with Natural Antimicrobial Agents in White Soft Cheese

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Abstract

Plainly treated foods are highly recommended in the recent world, so edible packaging using encapsulated essential oils in biopolymers have afforded a premium and nontoxic food preservation technique. In this work, chitosan-based emulsions made of liposomes loaded with thyme essential oil were explored to lengthen the shelf-life of white soft cheese. Chitosan-based emulsions with TEO/liposomes, prepared by 2% wt/v chitosan solutions (CL-TEO), are active for 60 days. Antimicrobial activities were assessed via determination of total viable counts of *Bacillus cereus* (*B. cereus*) and Enterococcus faecium (*E. faecium*) over 4 weeks at refrigerated storage at 5ºC. Cheese samples coated with CL-TEO 2% v/v and samples coated with CL-TEO 1% v/v plus nisin kept its satisfactory appearance, as well acceptable bacterial counts for 1 month at 5ºC. The results suggested that the formulated liposomal chitosan emulsions encapsulated TEO 2% v/v and liposomal chitosan emulsion encapsulated TEO 1% v/v plus nisin might be promising natural formulas to extend the shelf life in addition to preserving the flavour of white soft cheese.

KEYWORDS
B. cereus, *E. faecium*, white soft cheese, liposomal chitosan emulsion.

INTRODUCTION

Recently, the consumers worldwide favor food products free from chemical additives, therefore substitution with natural ingredients become a necessity (Weiss *et al*., 2015). Essential oils (EO) are among those natural alternatives which their use have many functional advantages. However, it is a technical fact that, adding essential oils as food additives usually modify the original taste of food (Hayouni *et al*., 2008). Furthermore, degradation of essential oils takes place by heating, or by contact with light through oxidation (El Asbahani *et al*., 2015).

Many advantages were attributed to essential oils encapsulation such as effective antimicrobial packaging that solve their strong odor problem (Al-Moghazy *et al*., 2020). Several research have been tested in this field (EL Azeem and Nada., 2015; Nada *et al*., 2018a), for instance, hydrogels (Nada *et al*., 2019a), nanofibers (Nada *et al*., 2019b; Abas *et al*., 2020), microspheres and composites. One of the promising materials is Natural polymers, they offer a great advantage to sustain the release of the Carried EO (Ibrahim *et al*., 2018). Although, a major disadvantage of these polymers, especially water-soluble chitosan and its derivatives, is the oil repulsion (Mohamed *et al*., 2020; Arafa *et al*., 2021). In order to solve this problem, attempt to decrease surface tension between the carrier and EO may aid in production of effective food coating solutions. Liposome, a lecithin phospholipid derivative, is a good approach to solve previous problem (Zahran *et al*., 2020) and an effective solution for hydrophobic materials encapsulation (Nada *et al*., 2018b). Numerous advantages have been linked to liposomes entrapping essential oils such as prolonged stability, EO protection from external deactivators and sustained release (Nada *et al*., 2016). Moreover, they augment the EO antibacterial activity (Gortzi *et al*., 2007; Liolios *et al*., 2009). Additionally, they slow down the EO release, thus, delaying the antimicrobial effect compared to the free essential oil (Engel *et al*., 2017). Moreover, the lesser mass of the oil droplets in nanoemulsions give a superior in situ antimicrobial concentration, in addition magnifying Brownian motion of smaller particles, simplifying the permeation of the oil droplets through bacterial barriers (Salvia-Trujillo *et al*., 2016).

Many reports have tried the essential oils incorporation in different food matrices as a preservative. Liposome encapsulated essential oils were successfully used in some types of hard cheese and minced meat (Cui *et al*., 2016a; Cui *et al*., 2016b; Khosravi-Darani *et al*., 2016; Lin *et al*., 2016; Cui *et al*., 2017).

Thyme, *Thymus vulgaris*, essential oil has been intensively tested and applied for its antimicrobial properties. On chromatographical analysis of thyme essential oil, many active compounds have been revealed, with carvacrol and thymol, were those having potent antimicrobial activity. Thyme nanoemulsion have prolonged the shelf life of ultra-filtrated labneh for maximum 6 weeks on cold storage, with acceptable flavor plus aroma owing

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to its high antibacterial effect on pathogenic and decomposition bacterial species (El-Sayed and El-Sayed, 2021).

Nisin is a leading bacteriocin created by definite species of *Lactococcus lactis* sp. lactis (Bonev *et al*., 2000; Jozala *et al*., 2009). Nisin has antimicrobial activity on Gram positive bacteria as Listeria, Staphylococcus, the of spores of *Bacillus*, besides *Clostridium* (Jozala *et al*., 2009). Nisin has been approved to be incorporated into processed cheese to hinder the spore's growth (Delves-Broughton 1990). Bacteriocins were used as a food biopreservatives that ruined by digestive proteases (Jeevaratnam, *et al*., 2005). Synergistic antimicrobial activity against foodborne pathogens were clear, when nisin was added to essential oils or essential oil constituents (Ettayebi *et al*., 2000; Olasupo *et al*., 2004), since nisin alone may has reduced antimicrobial activity in some dairy products with neutral pH (Gharsallaoui *et al*., 2016).

Nisin encapsulation and active packaging were developed to overcome these shortages. These technologies have been proved to eliminate *Bacillus cereus* in certain dairy products (Hanušova *et al*., 2010).

Mostly, the food microbial spoilage begins externally. Thus, if preventing the surface contamination is unattainable, its indispensable to protect food surface with specific an effective antimicrobial system (de Carvalho *et al*., 2015). Edible coating boosts the quality properties of food and act as a shield from environment.

In this study, we piloted combining Chitosan-coated liposomes loaded with 1% v/v TEO (CL-TEO 1%) v/v with nisin as antimicrobial coating in cheese. The main objective of this work was TEO nanoemulsion fabrication to extend shelf life of white soft cheese. Additionally, characterization of the antibacterial activity of CL-TEO 1% v/v (T1), CL-TEO 2% v/v (T2), Nisin (N) and liposomal chitosan emulsion encapsulated TEO 1% v/v plus nisin (T+N) against *B. cereus* and *E. faecium* in white soft cheese. In order to assess the suitability of the new coating, studying the organoleptic properties of cheese in control treatment during the cold storage period for four weeks at 5 ºC was done.

MATERIALS AND METHODS

Manufacturing of soft white cheese

Cheese samples were made using rennet coagulation method (Ahmed *et al*., 2021). Raw milk was initially pasteurized at 80°C for 10 min and cooled to 40°C. Microbial rennet was added according to the manufacturer's instructions, simultaneously with the addition of $CaCl_2$ (0.02%). After stirring, rennet-added milk was kept stable for around 60 minutes at 40°C. Curdled milk was cut into cubic pieces and transferred into sterile cheese cloth and initially hanged to allow whey drainage. Subsequently, pressing of cheese took place at 20°C. Cheese was kept at 5ºC for one month.

Bacterial Strain and Culture Condition

B. cereus and *E. faecium* were isolated from dairy products (Sakr *et al*., 2021). Bacterial strains were inoculated in a brain heart infusion (BHI) broth (HiMedia Laboratories Pvt. Ltd India) at 37ºC for almost 6 h, till getting the logarithmic phase cells for succeeding studies.

Preparation of the antimicrobials to the cheese samples

Stock solutions (10,000 IU/mL) of nisin (Beste Hellas, Athens, Greece, Nisaplin, activity of $1x10^6$ IU/g according to the manufacturer) were prepared in sterile distilled water just before addition. Nisin concentrations of 100 IU/g (N) were lastly added after making to the cheese samples, and these amounts are the same of those integrated by cheese producers (Davies *et al*., 1997).

Preparation of chitosan-TEO coating

Chitosan layer was formed by adding chitosan (2 g) medium molecular weight (Sigma- Aldrich, Germany) into 2% acetic acid solution. Constant stirring was continued until acquiring of clear solution. TEO was then added to previously prepared solution in two dissimilar concentrations (1 and 2% v/v). Final solutions were homogenized (12000 rpm for 2 min) by CAT Unidrive homogenizer.

Preparation of chitosan-liposomal coating solutions

Two solution concentrations were made, 1% and 2%, by adding 2 g lecithin (Lio, Turkey) to 0.5 and 1 g steam distillated TEO (supplied by oil extraction unit, National Research Centre, Egypt) and added to 50 mL of chloroform (Acros Organics, Belgium). At 22°C, solution was agitated for 30 min. after that 50 mL of chitosan/acetic was added. Subsequently, stirring was continued for another 30 min. at 60°C. Suspension turned milky and concentrated using compact pressure at 50°C for 2 h (Al-Moghazy *et al*., 2020). A concentration of 100 mg/l of nisin was dissolved in deionized water then added to 1% thyme essential oil concentration solution, then this solution was homogenized again at 10000 rpm for 2 minutes.

Application of bacterial culture, nanoemulsion and Nisin on to sliced white soft cheese

Prepared white soft cheese was hygienically cut into 4 × 4 cm squares. After cheese samples were inoculated by dipping in 10^6 CFU/mL bacteria culture for 10s, extra liquid was removed by a sterile cotton pad. Each cheese slice was placed in CL-TEO 1% v/v (T1), CL-TEO 2% v/v (T2), Nisin (N) and CL-TEO 1% v/v plus nisin (T+N), separately. Treated white soft cheese were stored for 4 weeks at 5ºC. Microbiological analyses were carried out at 0,1, 2, 3 and 4 weeks of storage. (Seydim *et al*., 2020).

Assessment of white soft cheese bacteriological quality during cold storage for four weeks

Descriptive 10 g of each sample was aseptically homogenized in 90 ml of a sterile 2% sodium citrate solution, in a stomacher for 1 minute. Ten-fold dilutions of the samples were prepared in 0.1% sterile peptone water (Oxoid, UK) and proper dilutions were used for enumeration as reported by Roberts and Greenwood (2003).

For counting of *B. cereus*, 0.1 mL of each dilution was inoculated onto duplicated agar plates containing *B. cereus* agar base (Oxoid, UK) supplemented with 100 mL/L of egg yolk emulsion (Oxoid, UK) and 5 mL/L of polymyxin B selective supplement (Oxoid, UK) as recorded by Owusu-Kwarteng *et al*. (2017).

While for *E. faecium*, Cheese samples (10 g) were homogenized in 90 mL of physiological sterile saline for 2 min in a blender 890-48H (Oster®/Sunbeam®, McMinnville, TN, USA). After filtering through sterile gauze, aliquots of 1 mL were taken, decimally diluted in physiological sterile saline (Caridi. 2003), and plated on Slanetz and Bartley agar plates (HiMedia, India) according to (Slanetz and Bartley 1957) guidelines. Colonies were counted and documented as log CFU/g.

Sensory evaluation of treated soft cheese

Samples of soft cheese were cut into about 5x5 cm pieces and placed on white plates. Samples were tempered at ambient temperature (20.0±2.0°C) and then presented to the panelists in a haphazard order. The cheeses were valued organoleptically at zero, 1, 2, 3 and 4 weeks of ripening in Dairy Control Department, Animal Health Research Institute by nine members of laboratory staff accustomed with white soft cheese. Panelists assessed cheese for appearance (10 points), body and texture (40 points) and flavor (50 points). Scores were acquired for the three sensory characteristics (Effat *et al*., 2012).

Statistical analysis

All assays were accomplished in triplicate. Statistics on a completely randomized design were performed with Two-way ANOVA procedure in the Statistical Package for Social Sciences (SPSS ver. 23) software. Duncan's multiple range test ($p<0.05$) was used to detect differences among mean values of white soft cheese properties in all test intervals.

RESULTS AND DISCUSSION

Microbiological quality of white soft cheese during cold storage for four weeks

White soft cheese is very popular product in Egypt. Though, raw milk and cheese are mostly considered as vehicles for transmission of pathogenic bacteria and are associated with worldwide outbreaks (Flowers *et al*., 1992). Furthermore, the pathogenic bacteria in cheeses negatively affect human health through increasing the sum of food poisoning cases and the severity of symptoms (Heikal *et al*., 2014). Contamination occurs due to the unsanitary surroundings during manufacture, storage, and handling as well as distribution (Kamal *et al*., 2017) leading to health risks and economic losses. (Youssef *et al*., 2018)

Aerobic spore-forming bacteria as *Bacillus* species hinder cheese manufacture through nitrate reduction as well as gas production in fermentative growth (Ternström *et al*., 1993). *B. cereus* is considered as poisonous organism, causing the "diarrheal syndrome" and the "emetic syndrome" (Arnesen *et al*., 2008). Total *B. cereus* counts of cheese samples for the control and all treatment groups verified about 2.4 log CFU/g at zero time for all treatments deprived of significant difference ($p<0.05$). Samples of C, N and T+N treatments were significantly the same along the first and second week of analysis, however, T1 and T2 were raised significantly ($p<0.05$). On the third week, T1, T2 and T+N displayed a significant ($p<0.05$) decrease in the total *B. cereus* counts. Furthermore, there were complete reduction of *B. cereus* in samples treated with T2, N and T+N, at the end of the fourth week of storage at 5°C (Fig. 1).

This study reflected the elevated antibacterial activity of T2, T+N, N inside the cheese matrix through decrease of the total *B. cereus* count at the end of storage period. In agreement with this study, Ahmed *et al*. (2021) described complete lessening of *S. aureus* at the end of first and second weeks of storage of thyme and ginger fortified cheese.

Several studies agree with the microbial inactivation efficiency of nisin and its combinations against *B. cereus*. Addition of nisin at 5 mg/kg inhibited *B. cereus* in processed cheese for 90 days of storage at 5ºC, (Plockova *et al*., 1996). Also, the inhibition of *B. cereus* in the packaged soft cheese using Polyethylene films coated by commercially available polyvinyldichloride (PVdC) containing both nisin and natamycin was reported byHanušova *et al*. (2010).

Table 1. Sensory evaluation of white soft cheese samples during four weeks of cold storage.

Assessed character	Storage period	Treatment type		
		C	T2	T+N
F (10)	0 day	7.333 ± 0.33^{a}	7.667 ± 0.33^{a}	7.333 ± 0.33^{a}
	1st week	7.00 ± 0.0^{a}	7.333 ± 0.33^{a}	6.667 ± 0.33^{ab}
	2nd week	5.667 ± 0.33^{b}	5.667 ± 0.33^{b}	5.667 ± 0.33^{b}
	3rd week	4.667 ± 0.33^{c}	4.333 ± 0.33^{c}	4.667 ± 0.33^{c}
	4th week	3.67 ± 0.33^{cd}	2.333 ± 0.33^{e}	3.333 ± 0.33^{d}
B&T (40)	0 day	36.333 ± 1.2^{a}	36.00 ± 1.15^{a}	36.333 ± 0.67^{a}
	1st week	35.67 ± 0.67^{a}	36.00 ± 1.15^{a}	35.33 ± 0.33^{ab}
	2nd week	31.33 ± 0.67^{c}	32.67 ± 1.2^{bc}	32.333 ± 1.2^{c}
	3rd week	28.0 ± 0.58^{d}	31.0 ± 0.58^{c}	25.333 ± 0.88^{d}
	4th week	$17.33^{e}\pm1.76$	17.33 ± 0.88^{e}	16.667 ± 0.67^{e}
C&A (50)	0 day	44.667 ± 1.2^{a}	44.667 ± 1.2^{a}	44.667 ± 1.2^{a}
	1st week	44.3 ± 0.33^{ab}	44.3 ± 0.33^{ab}	44.3 ± 0.33^{ab}
	2nd week	40.333 ± 1.2^{c}	40.333 ± 1.2^{c}	40.333 ± 1.2^{c}
	3rd week	40.33 ± 0.88^{c}	40.33 ± 0.88^{c}	40.33 ± 0.88^{c}
	4th week	41.0 ± 0.58^{bc}	41.0 ± 0.58^{bc}	41.0 ± 0.58^{bc}
Total (100)	0 day	88.333 ± 2.4^{a}	88.333 ± 2.4^{a}	88.333 ± 2.4^{a}
	1st week	88.0 ± 1.53^{ab}	88.0 ± 1.53^{ab}	88.0 ± 1.53^{ab}
	2nd week	88.667 ± 1.2^{a}	88.667 ± 1.2^{a}	88.667 ± 1.2^{a}
	3rd week	87.3 ± 0.33^{abc}	87.3 ± 0.33^{abc}	87.3 ± 0.33^{abc}
	4th week	87.6 ± 1.86^{abc}	87.6 ± 1.86^{abc}	87.6 ± 1.86^{abc}

F: Flavor; B&T: Body and Texture C&A: Color and Appearance

Data expressed as mean of three replicates. Means between columns (effect of treatments) showing the same capital letters are not significantly different ($p \leq 0.05$). Means between rows (effect of storage) showing the same small letters are not significantly different ($p \leq 0.05$). C= without coating; T2= Chitosan-coated liposomes loaded with 2% v/v TEO; T+N= Chitosan-coated liposomes loaded with 1% v/v TEO plus Nisin during storage at 5°C.

The antimicrobial activity of CL-TEO 1% (T1) was also prevalent, where *B. cereus* count reduced from 2.41 log CFU/g to 0.49 log CFU/g at the end of storage period at 5ºC. Similar records were attained for the inhibition of the total *B. cereus* count by about 3 log CFU/g by using EO of Zataria multiflora (3%) added to film-based basil seed gum (Gahruie *et al.* 2017). Nonetheless, Ayah and Saad (2016) found that thyme oil decreased *S. aureus* count in white soft cheese by about 2 $\log_{10}$ CFU/g at the end of the fourth week. These reductions in microbial count are mainly attributed to carvacrol and thymol antibacterial activity, damaging bacterial cells via changing the permeability of the cell membrane leading to escape of cell components (Ben Arfa *et al.*, 2006; Anžlovar *et al.*, 2014; Boskovic *et al.*, 2015).

Enterococci are dominant in milk and cheeses due to direct contamination by animals or indirect accumulation through water sources, milking equipment, storage tanks or their thermal resistance during cheese production (Foulquié Moreno *et al.*, 2006). They generate biogenic amines, antibiotic resistance, and biofilm (Kročko *et al.*, 2008). *E. faecium* of cheese origin reported as a multidrug resistant to Gentamicin high-level, Streptomycin high level, Clindamycin, Doxycycline and Tetracycline (Sakr *et al.*, 2021).

One week later of cooled storage, counts of the total *E. faecium* decreased in C and T1 treatments insignificantly compared to T2, N and T+N treatments where counts have reduced significantly ($p<0.05$). On the second week, total *E. faecium* counts has increased significantly ($p<0.05$) in C, T2 and N treatments without obvious differences among them. While T1 increased and T+N decreased but insignificantly. However, *E. faecium* count in the control and all treatments in the third week was insignificantly decrease. However, it diminished significantly ($p<0.05$) in T1. On the fourth week, the total *E. faecium* counts has amplified insignificantly through the entire treatments, but the increase in C was significant. Though there was insignificant decrease in *E. faecium* count in samples treated with N (Fig. 2).

As well, the present study revealed that *E. faecium* total count decreased by 1 log CFU/g in T1 and T2 treated cheeses at the end of storage period at 5°C as found by Han *et al.* (2015) who recorded 1 log CFU/g lactic acid bacteria LAB in thyme oil treated mozzarella cheese lower than untreated cheese samples at day 20 at 4 ºC.

Also, Selim (2011) informed that addition of thyme oil at concentration of 0.5 % and 1 % initiated significant drop in the growth rate of vancomycin resistant enterococci in cheese and meat at 7ºC. Furthermore, Moghimi *et al.* (2017) recorded robust antimicrobial effect of hydroxy propyl methylcellulose (HPMC) with nanoemulsions of Thymus daenensis EO against *E. faecium*.

Also, Nostro and Papalia (2012) have described Thyme EO antimicrobial mechanism of action against E. hirae, where carvacrol is a monoterpenoid phenol interacts with the fatty acid chains of the lipid bilayer leading to destabilization and collapse of the membrane. Then it degenerates the proton motive force and exhaust the ATP pool.

The obtained results revealed, limited antimicrobial activity of nisin, where *E. faecium* increased from 2.88 log CFU/ml at 0 week to 3.25 log CFU/ml at the termination of the fourth week. The reduced activity of nisin to certain dairy products is well-known (Gharsallaoui *et al.*, 2016), particularly dairy products

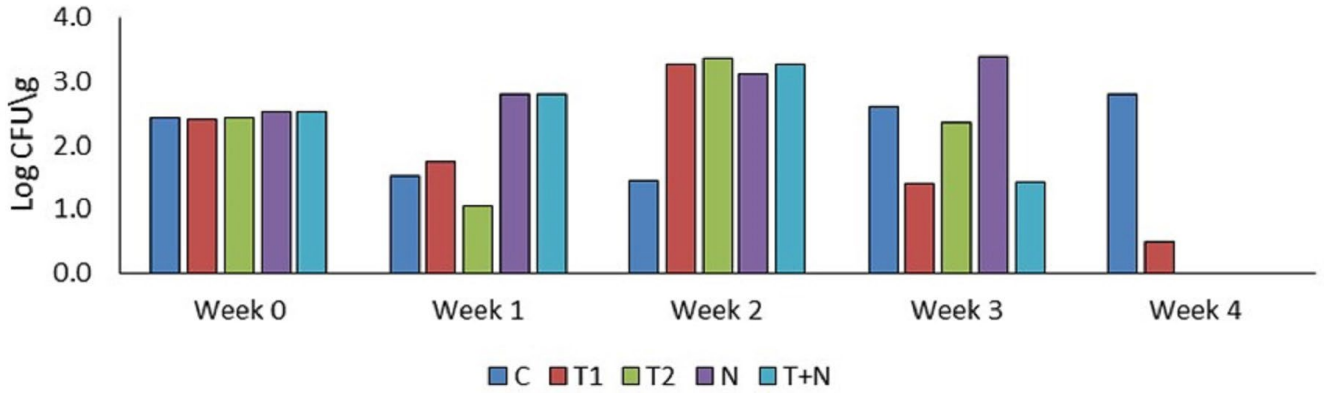

Fig. 1. Total B. cereus counts of un/treated white soft cheese samples over 4 weeks: C= without coating; T1= Chitosan-coated liposomes loaded with 1% v/v TEO; T2= Chitosan-coated liposomes loaded with 2% v/v TEO; N= Nisin; T+N= Chitosan-coated liposomes loaded with 1% v/v TEO plus Nisin during storage at 5 ºC.

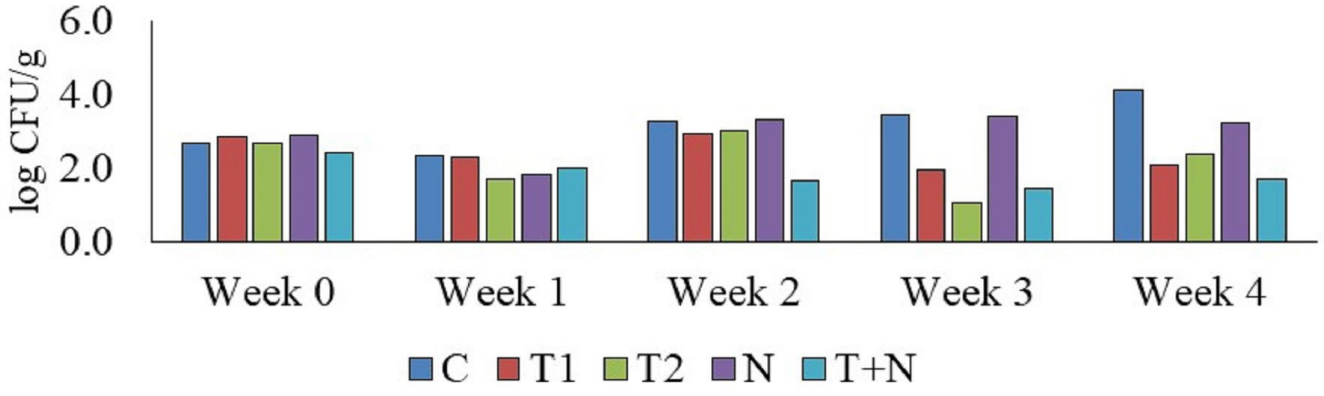

Fig. 2. Total E. faecium counts of un/treated white soft cheese samples over 4 weeks: C= without coating; T1= Chitosan-coated liposomes loaded with 1% v/v TEO; T2= Chitosan-coated liposomes loaded with 2% v/v TEO; N= Nisin; T+N= Chitosan-coated liposomes loaded with 1% v/v TEO plus Nisin during storage at 5 °C.

with a neutral pH prepared from whole milk (Sobrino-Lopez and Martin-Belloso, 2008; Gharsallaoui *et al*., 2016). For instance, the addition of 250 µg/g nisin to Queso Fresco, a fresh cheese that has a neutral pH and is formed from whole milk, affords L. monocytogenes control for just a few days (Van Tassell *et al*., 2015; Ibarra-Sanchez *et al*., 2018; Feng *et al*., 2019). Numerous aspects have been suggested to elucidate nisin limits in certain dairy foodstuffs including: nisin stability shortage at neutral pH, nisin probability to barrier to fat globules, cationic nisin binding to anionic casein, existence of divalent cations hindering entrance to cell membrane phospholipids, and nisin resistance progress possibility.

Strategies to improve nisin antimicrobial activity in cheese are designated including combination with encapsulated thyme oil in liposomes and coated with chitosan in the form of T+N.

Nisin has shown synergy when combined with CL-TEO 1% v/v (T1) to reduce the populations of *E. faecium* count from 2.44 log CFU/ml at 0 week to 1.69 log CFU/ml at the end of the storage period compared with Mei *et al*. (2015) who exposed that an edible chitosan-starch-based coating fortified with pine needle essential oil, Cornus officinalis fruit extract, and nisin as antimicrobial agents applied on Bod Ljong cheese decreased aerobic plate count throughout storage for 25 days.

Sensory evaluation of white soft cheese during cold storage for four weeks

The scores documented for flavor, body and texture, color and appearance, and total acceptability demonstrated that adding of T2 and T+N to white soft cheese slightly affected the sensory characteristics (Table 1).

Flavor scores of white soft cheese treatments faintly decreased in T2 and T+N treatments, whereas during storage flavor of T2 treated cheese was significantly ($p<0.05$) declined at the fourth week. Control, T2 and T+N averagely displayed acceptable and good flavor. Actually, various studies acclaimed the restricted use of EOs since their impact on organoleptic properties of cheese mainly their flavor, where flavor of thyme oil fortified soft cheese scored 35 until the termination of second week then 32 in the third week, as well as 29 in the fourth week of storage with a little bitter almond flavor (Samah and Ahmed, 2019). Moreover, Han *et al*. (2015) reported that thyme oil treatment significantly ($p<0.05$) affected the flavor of shredded leading to less preferred flavor, compared with control, so this study revealed the advantage of essential oil encapsulation in keeping acceptable flavor of cheese.

Among the tested sensory attributes, body and texture varied marginally between the entire treatments besides through the storage period. The uppermost values of body and texture were recorded in the T2 samples, followed by C and T+N along the four weeks of storage period. Results of El-Sayed and El-Sayed (2021) were in accordance with the present study, whereas the body and texture of UF labneh was faintly affected by EO treatments.

Regarding color and appearance, there was a significant difference ($p<0.05$) in the mean scores throughout the second, the third in addition to the fourth weeks of storage period. The control samples had a clean natural white color furthermore; other treated samples existed with the same color, without affecting the significant difference as shown in Table 1. This is in accordance with mean scores of thyme oil treated mozzarella cheese, where the color of cheese hasn't affected significantly ($p>0.05$). On the other hand, there was homogeneity and velvety feeling in color and appearance scores in labneh treated by TEO nanoemulsion (El-Sayed and El-Sayed, 2021).

In this work, the overall acceptability slightly affected along the storage period, even if the white soft cheese treated with T2, or T+N. In accordance, laboratory manufactured fresh soft cheese fortified with 0.01% ginger and thyme oils scored grade A regarding overall sensory acceptability during the storage period for 1 month at 4ºC with the highest scores in the first two weeks (Ahmed *et al*., 2021). Conversely, Ayah and Saad (2016) stated that cheese fortified with thyme oil recorded grade B until the end of the second week then grade C on the third and fourth weeks relative to the overall acceptability.

CONCLUSION

Recent food science commended using natural antimicrobial as a preservative for expanding the shelf life of various foodstuffs. Founded on this study, it can be settled that *T. vulgaris* essential oils made-up in nanoemulsion form bared antimicrobial influence against *B. cereus* and *E. faecium* strains. Additionally, adding of T2 and T+N to white soft cheese as a preservative has insignificant influence on sensory properties. CL-TEO 2% v/v (T2) and CL-TEO 1% v/v (T1) plus nisin have presented to extend the shelf life up to 4 weeks at 5ºC of white soft cheese, keeping acceptable taste and aroma.

CONFLICT OF INTEREST

The authors declare that they have no competing financial interests or personal relations that could have seemed to affect the work described in this paper.

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